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## Towards an integrated view of HCN channel role in epilepsy

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### Abstract

Epilepsy is the third most common brain disorder and affects millions of people. Epilepsy is characterized by the occurrence of spontaneous seizures, i.e., bursts of synchronous firing of large populations of neurons. These are believed to result from abnormal regulation of neuronal excitability that favors hypersynchrony. Among the intrinsic conductances that govern neuronal excitability, the hyperpolarization-activated current ( $I_h$ ) plays complex and important roles in the fine-tuning of both cellular and network activity. Not surprisingly, *dys*regulation of  $I_h$  and/or of its conducting ion-channels (HCN) has been strongly implicated in various experimental models of epilepsy, as well as in human epilepsy. Here we provide an overview of recent findings on the distinct physiological roles played by  $I_h$  in specific contexts, and the cellular mechanisms that underlie these functions, including the subunit make-up of the channels. We further discuss current knowledge of dysregulation of  $I_h$  and HCN channels in epilepsy in light of the multifaceted functions of  $I_h$  in the brain.

### The h-current ( $I_h$ ): a versatile regulator of cellular and network excitability

Among the many ionic conductances that shape neuronal excitability, the hyperpolarization-activated current  $I_h$  has long been a subject of interest and debate among researchers. Unlike other voltage-gated currents,  $I_h$  is activated upon relative *hyper*-polarization of the cell membrane. Conducted by a mixed cationic current with reversal potential values around -30 mV,  $I_h$  activation counteracts the hyperpolarization that triggered it [1,2]. Because  $I_h$  is partially activated at physiological, “resting” conditions, it provides constant depolarization of the membrane potential [3-5]. Thus,  $I_h$  is thought to function as a stabilizing negative-feedback loop that responds to alterations in membrane potential [1,2]. Alongside its depolarizing role,  $I_h$  exerts a shunting effect on excitable cells: being open at subthreshold potentials,  $I_h$  reduces the input resistance of the membrane ( $R_{in}$ ), thus dampening the ability of incoming inputs to alter membrane voltage.

The net effect of the depolarizing and shunting properties of  $I_h$  on excitability is combinatorial and depends on many factors (Figure 1), as illustrated by the roles of  $I_h$  in

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CA1 hippocampal pyramidal neurons. In these neurons, the density of  $I_h$  along apical dendrites increases with the distance from the soma [6]. A physiological consequence of this heterogeneous distribution (mediated by the effect of  $I_h$  on the input resistance and membrane time constant) is that EPSP time course is increasingly shortened with the distance from the soma [6]. As a result, temporal summation of synaptic inputs at the soma is similar regardless of whether these inputs occur at proximal or distal sites [6] (but see [7]). Another physiological action of  $I_h$ , mediated by its tonic depolarizing effect, is to increase steady-state inactivation of low-threshold, voltage-gated calcium channels [8], and thus restrict the genesis of dendritic calcium spikes. The effects of  $I_h$  on excitability involve interactions with other intrinsic conductances: whereas  $I_h$  increases the peak voltage amplitude of *weak* EPSPs, it inhibits the peak amplitude of responses to *strong* stimuli, and the net effect on EPSPs depends upon an intricate interaction between the  $I_h$ -mediated tonic depolarization and the  $K^+$ -mediated conductance,  $I_M$  [9]. Regulation of synaptic signaling by  $I_h$  is not limited to excitatory inputs.  $I_h$  suppresses inhibitory (GABA<sub>A</sub>-mediated) post-synaptic potentials (IPSPs) through interactions with other active conductances and cellular passive electric properties [10,11]. The relative depolarization generated by  $I_h$  in distal dendrites can alter GABA<sub>A</sub> signaling from shunting to hyperpolarizing, and thus shortens the time-window for coincidence-detection [12].

From the above, it is clear that  $I_h$  has multiple effects in regulating dendritic excitability, including through interactions with other intrinsic conductances. Yet how do these different effects of  $I_h$  on inhibitory, excitatory and intrinsic signals integrate in a physiological context? This question was recently addressed in subthalamic nucleus (STN) neurons [13]. In these neurons (which express different HCN channel subunits compared with CA1 pyramidal neurons), dendritic  $I_h$  was activated only upon strong hyperpolarizing input onto the dendrite, and thus served a homeostatic role in counteracting GABA<sub>A</sub>-mediated signals. In an analogous manner to that described in CA1 pyramidal neurons [8], activation of  $I_h$  by inhibitory input facilitated steady-state inactivation of low-threshold voltage-gated calcium channels, leading to inhibition of dendritic calcium spikes. Whereas theoretically  $I_h$  could suppress also the temporal summation of *excitatory* inputs, the authors demonstrated that the same inhibitory input that activated  $I_h$  shunted the effects of  $I_h$  on EPSP summation, and concluded that, in STN neuronal dendrites,  $I_h$  selectively regulated inhibitory signaling [13].

$I_h$  is not limited to somato-dendritic subcellular distribution, and presynaptic  $I_h$  has been reported in various classes of neurons [14-17]. The presynaptic functions of  $I_h$  in mammalian brain have remained elusive [1, 16], but, in axon terminals of layer 3 entorhinal cortex neurons,  $I_h$ -mediated depolarization was recently found to restrict the activity of T-type  $Ca^{2+}$  channels, leading to reduced calcium influx and inhibition of synaptic release [17]. It remains to be seen whether similar mechanisms exist in presynaptic terminals of *interneurons* [14,15] or whether  $I_h$  serves an opposite, facilitatory role in these neuronal populations [14].

In addition to its roles in dendritic integration, membrane potential stabilization and regulation of synaptic transmission,  $I_h$  is an important modulator of oscillatory activity at both cellular and network levels. At cellular levels,  $I_h$  is critical for theta resonance (the preferential response of a neuron to oscillating inputs at specific frequencies). The slow kinetics and sub-threshold activation of  $I_h$  enable this current to filter out inputs at low frequencies (<3 Hz). This high-pass filtering property, in combination with low-pass filtering properties provided by the membrane capacitance, render cells with a strong  $I_h$  particularly responsive to inputs in the theta range (3-12 Hz) [18,19], a property also found in specific types of interneurons [20]. In cell-types with high  $I_h$  density at distal dendrites, dendritic  $I_h$ -dependent resonance is more pronounced, and preferentially filters signals that propagate from the soma to dendrites [19]. The filtering properties of  $I_h$  may contribute to

regulating *in vivo* theta rhythms [4], and other network oscillations (e.g., delta and gamma) and rhythmic firing (reviewed by [1]).

## **$I_h$ diversity originates from exquisite control of HCN channels at multiple levels and enables physiological plasticity**

The functional diversity and multiple roles of  $I_h$  in different physiological settings (discussed above) require tight regulation of the pore-forming proteins that conduct  $I_h$ , namely, HCN channels. HCN channel regulation involves many processes that take place at three levels: (1) regulation of the biophysical properties of the channels (such as voltage-activation profile and kinetics); (2) regulation of the number of channels expressed on the plasma membrane; and (3) localization of the channels to distinct subcellular compartments.

A critical determinant of the biophysical properties of HCN channels is their molecular make-up. Four different channel isoforms exist (HCN1-4) that can assemble in different combinations to yield homo- or hetero-tetrameric complexes with different properties [1,2,21]. Indeed, variability in  $I_h$  properties among cell populations and during different developmental stages is often associated with distinct expression profiles of specific HCN channel isoforms [22-25]. The conductive properties of HCN channels are further diversified by discrete interactions with other molecules, notably cyclic AMP (cAMP): cAMP binds a sequence on the C terminus of the channel, and influences HCN channel function by accelerating its kinetics and shifting its voltage-activation curve to more depolarized values [1,2]. The sensitivity of HCN channels to cAMP is isoform-specific: HCN4 > HCN2 >> HCN1 [1,2]. Thus, isoform-specific interaction of HCN channels with regulating molecules provides another dimension to the diversity of  $I_h$ . HCN channel function is modified by other ‘small’ molecules such as PIP(2) [26,27], as well as by interacting/auxiliary proteins (reviewed in [28]). Finally, phosphorylation of different HCN channel isoforms at multiple sites influences their biophysical properties (e.g. [29-31]).

The *number* of surface-expressed HCN channels influences the magnitude and properties of  $I_h$  (via changes in relative abundance of the constituents of the channel). Mechanisms controlling the number of surface HCN channels act at different time-scales. At a relatively long time scale (hours to days), transcriptional regulation determines the total amount of HCN channel protein in the cell [1]. This regulation is isoform-specific, acts via specific transcription factors [32], and depends on network activity [33]. Post-translational mechanisms also modulate HCN channels. For example, these channels are heavily glycosylated in the mammalian brain [34,35] and glycosylation influences both the total number of HCN channels in the membrane and their heteromerization (and hence properties) in a subunit-specific manner [35,36]. Modulation of HCN channel surface expression at shorter time-scales can occur via local regulation of channel membrane insertion, internalization and recycling [37,38]. Importantly, the dynamics of HCN channel trafficking and surface expression are activity-dependent [38], with implications to neuroplasticity and disease. Auxiliary proteins interact with HCN channels to regulate their surface expression: a family of splice variants of TRIP8b (an auxiliary protein that interacts directly with HCN channels) can either up- or down-regulate HCN channel surface expression [39,40]. Other candidate auxiliary proteins have been implicated in surface regulation of HCN channels (reviewed in [28]).

The targeting of HCN channels to distinct sub-cellular domains influences their location-dependent roles in regulation of excitability. Distinct distribution patterns of HCN channels exist in somata, dendrites and axons of specific cell types and brain regions, as well as during development [16,17,23,41,42]. Whereas ample information exists on the distribution of HCN channels, little is known of the molecular mechanisms that underlie their targeting

to sub-cellular domains. Time-lapse live imaging in hippocampal neurons indicates that vesicular trafficking dynamics of HCN channels are isoform-specific [38], and neuronal activity influences both trafficking and long-term subcellular distribution [38,43]. Because the HCN-interacting protein TRIP8b colocalizes with HCN1 channels and the distribution of the protein is disrupted in HCN1 knockout mice, TRIP8b is a candidate for dendritic targeting of HCN channels [44,45]. In addition to a heterogeneous distribution in the different cell compartments, HCN channels appear to be differently distributed within the same brain region. Thus,  $I_h$  decreases along the dorsal-ventral axis in entorhinal cortex stellate cells, providing these neurons with different integrative properties [46,47].

Together, the diverse regulatory mechanisms described above enable exquisite functional plasticity of  $I_h$ , such as seen during development [16, 22] or when synaptic plasticity is triggered [18,38]. When the mechanisms of HCN channel plasticity are disrupted, the resulting dysregulation of  $I_h$  might contribute to neurological disorders including epilepsy.

### (3) HCN channel dysregulation in epilepsy: the wrong amount in the wrong place, at the wrong time

Since the original implication of HCN channels in epilepsy in 2001 [48], many studies have linked these channels to the epileptogenic process. In resected hippocampi from patients with temporal-lobe (limbic) epilepsy, enhanced levels of HCN1 channel expression and dendritic localization were found in granule cells of the dentate gyrus [49], and recent work has identified a mutation in the *HCN2* gene and augmentation of  $I_h$  in patients with genetic epilepsy with febrile seizures plus (GEFS+) [50]. Deletion of the *HCN1* gene in mice results in increased excitability and seizure susceptibility [51,52], and reduction or deletion of the *HCN2* isoform leads to spontaneous ‘absence’ seizures [53,54].

In accord with the diverse regulatory mechanisms and versatile functions of  $I_h$  in the normal brain, the dysregulation of  $I_h$  and HCN channels in epilepsy is dynamic and intricate (Figure 2). HCN channel abnormalities in the epileptic brain can manifest as altered mRNA and protein expression [55–60], sub-cellular distribution [44,61] or biophysical properties [48,58,62]. The causal relationship between aberrant HCN channel regulation and the epileptic process is further complicated by the fact that alterations in HCN channel expression, localization and function vary across animal models of epilepsy. Both early and late changes affecting diverse isoforms in distinct spatial patterns have been reported. For example, in the pilocarpine model, progressive reduction in HCN1 and HCN2 protein levels results in diminished  $I_h$  amplitude in dendrites of CA1 pyramidal neurons, leading to the disruption of theta resonance [58,63]. In contrast, following hyperthermia-induced seizures, the same population of cells exhibit *enhanced* dendritic  $I_h$  accompanied by altered gating properties [48,62], likely mediated by isoform-specific transcriptional regulation and increased HCN1/HCN2 heteromerization [56]. These seemingly conflicting results from different animal models demonstrate that augmented  $I_h$  can be associated with both increased and decreased excitability, depending on the physiological context [64], and potential interaction with other conductances.

The *temporal* patterns of HCN dysregulation in the epileptic brain are complex, and both transient and long-lasting alterations in hippocampal HCN/  $I_h$  have been reported in pilocarpine, KA and febrile seizures models [55,56,58,60,63]. Interestingly, alterations in  $I_h$  gating properties in CA1 pyramidal neurons were reported within hours following induction of seizure-like-activity *in vitro*, attributable to activation of the phosphatase calcineurin and inhibition of p38 MAPK [65].

Spatial selectivity of HCN-channel pathology further contributes to the complex involvement of  $I_h$  in epilepsy. In acquired hippocampal epilepsy, HCN channel expression levels vary across different hippocampal regions in isoform-specific and region-specific fashions [55,57]. Region-specific alterations have been found in the WAG/Rij rat model of genetic absence epilepsy, where *reduced* HCN1 channel expression in layer 5 cortical neurons was reported to increase excitability via enhanced calcium electrogenesis [66], while *increased* HCN1 levels in thalamocortical neurons impaired their firing pattern via reducing the responsiveness of  $I_h$  to cAMP [59]. Reduced response of  $I_h$  to cAMP (triggered by imbalance of HCN subunit expression) was found also in the GAERS rat model of absence epilepsy [25].

Epilepsy may also involve abnormal subcellular distribution of HCN channels. Dendritic HCN1 localization was augmented in hippocampi resected from patients with epilepsy [49]. In animal models, transient upregulation of HCN1 surface expression occurred in CA1 pyramidal neurons 1-2 days after an epilepsy-provoking insult, followed by a *downregulation* 4 weeks later with mislocalization of the channels from distal dendritic domains to somata [44]. Recent work in freeze-lesion models of cortical dysplasia-provoked epilepsy found reduced HCN1 channel presence in distal dendrites of layer 5 cortical neurons [61].

In summary, multiple mechanisms, including transcriptional control, trafficking and channel modification act at different temporal and spatial scales to modulate  $I_h$  in the epileptic brain. In general, such changes might contribute to epileptogenesis, i.e., ‘cause’ epilepsy, or be a result of the epilepsy. The occurrence of several of these HCN changes early after the insult that triggers epilepsy, and/ or prior to the onset of spontaneous seizures [32,55,66] suggests a causal role. To fully grasp the contribution of these alterations to the epileptogenic process, physiological, molecular and cellular approaches should be integrated (Box 1). Recent discoveries have greatly advanced our understanding of the protean roles that  $I_h$  plays in the normal brain in different contexts, and their underlying cellular mechanisms. These, and future studies, will ultimately lead to an integrated view on the role of HCN channel pathology in epilepsy, at levels of analysis spanning single molecules to cellular and network domains. This understanding, in turn, will promote the design of new strategies for better treatment of the epilepsies.

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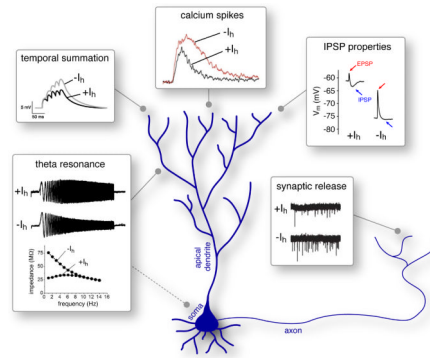
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**BOX 1. Factors that influence the potential contribution of HCN channel dysregulation to hyperexcitability and epilepsy**

- **Brain region and neuronal population:** In what cell populations are  $I_h$ /HCN channels altered? (Principal cells? Interneurons? Which brain region?); What is the specific role of  $I_h$  in this population and how does it influence network excitability?
- **Sub-cellular distribution:** Do changes in  $I_h$ /HCN channels take place in dendrites, soma, axon terminals?
- **Quantitative properties:** Is  $I_h$  upregulated or reduced?
- **Qualitative properties:** How is the molecular makeup of the channels altered? How are biophysical properties changed (voltage-activation, kinetics, cAMP gating)?
- **Temporal pattern:** Are changes in  $I_h$  dynamic or static? Activity dependent? Are they a result of other cellular and network changes?
- **Interplay with active and passive properties:** Are  $I_h$ /HCN changes accompanied by changes in other intrinsic conductances, synaptic inputs or the passive properties of the cell? Do changes in  $I_h$  lead to alterations of other channels?

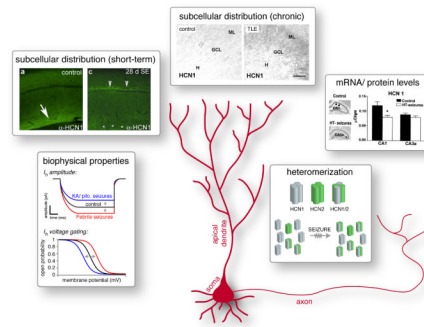
### Highlights

- The hyperpolarization-activated current  $I_h$  plays complex and important roles in the fine-tuning of both cellular and network activity
- The distinct physiological roles of  $I_h$  depend on factors including HCN subunit composition, interacting proteins and molecules, subcellular localization and the context of synaptic activity
- $I_h$  dysregulation in epilepsy occurs at multiple regulatory levels and time frames, with complex effects on neuronal excitability
- A full understanding of the physiological and pathological roles of  $I_h$  will clarify the role of HCN channels as molecular targets in epilepsy therapy



**Figure 1. The multiple functions of  $I_h$  on neuronal activity are context-and location driven**

A schematized neuron depicting principal functions of  $I_h$  in distinct subcellular domains. In dendrites,  $I_h$  reduces dendritic summation (inset from [1], with permission); the current also represses dendritic calcium spikes (inset from [8], with permission), and converts the effect of IPSPs on the membrane potential from shunting to hyperpolarizing (inset modified from [12], with permission). In axon terminals,  $I_h$  reduces synaptic release through interaction with calcium channels (inset from [17], with permission). In both soma and dendrites,  $I_h$  augments theta-frequency resonance (inset modified from [19], with permission). In neurons that possess increased dendritic  $I_h$  density, the effects on theta-frequency resonance are more pronounced in dendrites compared to the soma.



### Figure 2. Abnormal HCN channel regulation and function in epilepsy

Dysregulation of HCN channels/  $I_h$  in epilepsy occurs at multiple levels. Seizure-induced alterations of the biophysical properties of  $I_h$  include either up- or down- regulation of current amplitude and modification of gating. The sub-cellular distribution of HCN channels along the somato-dendritic axis is altered both short-term, e.g., in hippocampal CA1 neurons in the pilocarpine model (insert modified, with permission, from [44]), and in dentate gyrus granule cells of human epileptic patients (insert from [49], with permission). Seizure-induced alterations in HCN channel mRNA and protein levels occur in many epilepsy models, and are both region- and channel isoform- specific (insert from [55], with permission). Altered synthesis of specific HCN channel isoforms may drive, at least in part, the increased, seizure-induced HCN1/2 heteromerization in hippocampus [55,56].